

Stimulation of nitrogen fixation in refractory organic sediments by *Caulerpa taxifolia* (Chlorophyta)

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Abstract

Estimates of N₂ fixation by substrata associated with the rhizophytic alga *Caulerpa taxifolia* were obtained using the acetylene reduction method. On average, growth of *C. taxifolia* enhanced rates of acetylene reduction in underlying dead sea grass sediments by a factor of 28, although the degree of enhancement was variable. The average rate of ethylene production was 3.96 nmol cm⁻³ h⁻¹ (range = 2.5–6.2 nmol cm⁻³ h⁻¹). There was no apparent stimulation of N₂ fixation in substrata collected close to a wastewater outlet. *C. taxifolia* appears to enhance N₂ fixation by releasing photosynthetic product into the rhizosphere, mimicking the behavior of saltwater vascular plants. The excreted organic C activates the fermenting bacterial community, which in turn makes substrates available to the sulfate reducers. The associated microbial reactions create strong reducing conditions that favor N₂ fixation, of which many sulfate reducers are capable. Nitrogen fixation can serve to reduce the N deficit that inhibits bacterial decomposition of refractory sea grass waste, thereby enhancing organic matter turnover and nutrient supply to the alga's rhizoids. This process likely assists *C. taxifolia* to proliferate upon refractory organic sediments in low-nutrient seawater.

The green alga *Caulerpa* produces a pseudo-root system that functions to anchor the plant, as well as to take up nutrients (Williams 1984; Chisholm et al. 1996). Nutrient uptake from the substratum potentially enables the alga to proliferate in oligotrophic seawater on eutrophicated substrata (Chisholm et al. 1996). Biogeochemical studies in the northwest Mediterranean have indicated a link between the proliferation of *Caulerpa taxifolia* Vahl. C. (Agardh) and the availability of nutrients discharged in urban wastewater or resulting from the decay of sea grass vegetation (Chisholm et al. 1997). Association with wastewater pollution is not surprising because algal production has often been shown to increase with nutrient discharge. Association with decomposition of sea grass vegetation is less straightforward because sea grass tissues generally have low N content relative to C, which limits bacterial nutrient turnover (Goldman et al. 1987; Paerl 1990).

Field and laboratory observations demonstrate that sediments containing dead sea grass matter frequently turn black in color after penetration by the rhizoids of *C. taxifolia* (Fig. 1). The black coloration is due to bacterial reduction of sulfate to sulfide, indicating strong reducing conditions that favor N₂ fixation. Nitrogen fixation could alleviate barriers to organic decomposition (Paerl 1990; Tibbles et al. 1994), thus enhancing nutrient supply to the alga's rhizoids. *C. taxifolia*

appears to activate the fermenting bacterial community into delivering secondary substrates to the sulfate reducers by releasing photosynthetic product from its rhizoids, as do salt marsh and sea grasses from their root systems (e.g., Pollard and Moriarty 1991; Welsh et al. 1997; Blaabjerg et al. 1998).

Materials and methods

We postulated that penetration of dead sea grass sediments by the rhizoids of *C. taxifolia* could enhance rates of N₂ fixation relative to uncolonized sea grass sediments and sediments fertilized by an external source of inorganic nitrogen. To test these postulates, we obtained sediments with and without *C. taxifolia* cover by SCUBA diving, on 1 October 2001 from (1) a dead bed of the sea grass *Posidonia oceanica* L. Delile near Cap Martin in southeast France and (2) close to the pretreatment and main sewer outlets for the Principality of Monaco (Fig. 2). Surface water samples collected at site 2 between July 2000 and July 2001 contained, on average, 4.2 μmol NO₃⁻ L⁻¹, 0.18 μmol NO₂⁻ L⁻¹, and 0.44 μmol NH₄⁺ L⁻¹ and maximum concentrations of 10.7 μmol NO₃⁻ L⁻¹, 0.40 μmol NO₂⁻ L⁻¹, and 1.44 μmol NH₄⁺ L⁻¹ (RNO 2001).

All samples were gathered at a depth of 10 m by hand to minimize disturbance to the alga's rhizosphere. At each site, approximately equal volumes of sediment (3,000–4,000 cm³) and associated *C. taxifolia* tissue were collected from three locations, separated by distances of at least 5 m to avoid sampling sediments linked by the same algal stolons (maximum stolon length = 2.8 m; see Meinesz et al. 1995). Uncolonized sediments were sampled in the same manner. Sediments were sampled from the sediment horizon to a depth of 10–15 cm. The samples were transported in plastic containers to the laboratory in Monaco and transferred into four 60-L flow-through aquaria within 3 h of collection.

Two of the four aquaria were used to cultivate samples from each collection site on a 10–15-cm-deep basal layer of

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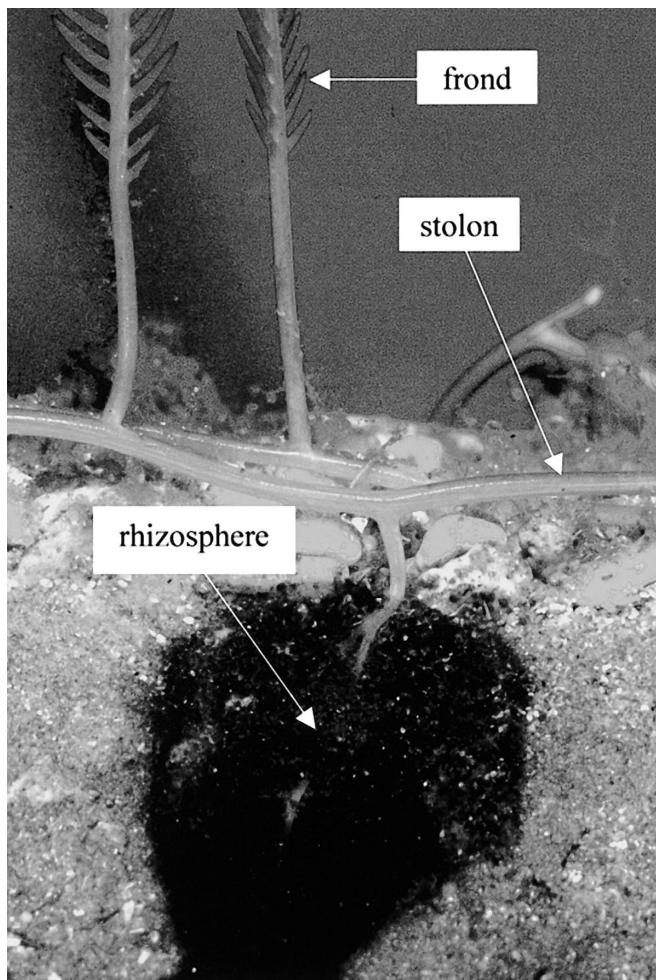


Fig. 1. Black zone in sediments surrounding the rhizoids of *Caulerpa taxifolia* caused by bacterial reduction of sulfate to sulfide.

their original substratum. Each aquarium thus comprised sediments taken from each of the three intrasite locations, half of which bore cover of *C. taxifolia* and half of which did not. By maintaining colonized and uncolonized sediments in the same aquarium, it was possible to eliminate extraneous environmental variables and focus upon differences caused by the presence or absence of *C. taxifolia*, except, that is, for differences among the sediment and algal samples themselves. The other two aquaria were used to cultivate free-living *C. taxifolia* samples from each collection site in the absence of any basal substratum, after their rhizoids had been thoroughly cleaned of adhering sediment.

All of the aquaria were fed continuously with fresh, filtered (2.5 μm) seawater, pumped to the laboratory from a depth of 50 m in the Mediterranean and heated to $22 \pm 0.5^\circ\text{C}$ using thermostatically controlled immersion elements (1,000 W). Samples were grown under $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ of white fluorescent light (6,000 K color temperature) on a 14:10 light:dark photoperiod for 4 weeks prior to experiments and then for the 4 weeks that it took to perform the acetylene reduction assays.

Incubations to determine rates of acetylene reduction were

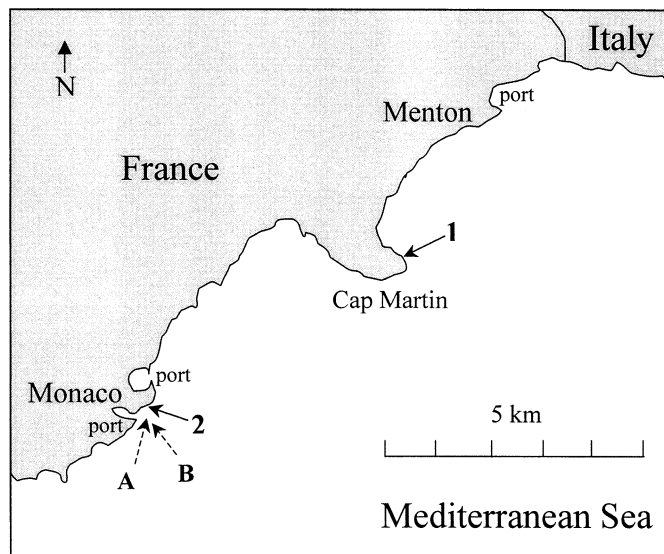


Fig. 2. Sample collection sites: (1) a dead bed of the sea grass *Posidonia oceanica*; (2) adjacent to the Monaco wastewater pre-treatment (A) and main (B) sewer outlets, whose locations are indicated by broken arrows.

carried out in wide-mouthed, clear glass jars placed on an agitating tray in a thermostatically controlled water bath. The bath was adjusted to the cultivation temperature. Each jar had an internal volume of 600 ml and possessed a clip-down, airtight lid. A 10-mm-diameter hole was cut in the lid and plugged with a rubber septum. The septum was positioned at the periphery of each lid to minimize shading of the samples. Algae, sediment, and algal-sediment associations were placed in the jars according to designated treatments, and volumes of seawater were added to leave gas headspace volumes of 250 ml. Blanks were prepared that contained seawater only.

Purified acetylene was injected through the rubber septa using sterile syringes fitted with 0.6-mm-diameter needles to make up 20% of the gas phase (see Capone 1993). Samples were incubated for 4 h under $75 \mu\text{mol m}^{-2} \text{s}^{-1}$ of artificial daylight supplied by a 400-W overhead metal halide lamp. The reduced irradiance relative to the preassay conditions ($100 \mu\text{mol m}^{-2} \text{s}^{-1}$) was enforced by space constraints in the culture cabinet in which incubations were performed. That is, a smaller lamp needed to be fitted, which could deliver a maximum of $75 \mu\text{mol m}^{-2} \text{s}^{-1}$ when arranged to provide even illumination over the experimental subjects. Although $75 \mu\text{mol m}^{-2} \text{s}^{-1}$ is generally less than samples receive at a depth of 10 m, it is closer to the average irradiance received by the species over its depth range in the northern Mediterranean (near surface to 99 m; see Belsher and Meinesz 1995).

During incubations, 550–600- μl samples of headspace gas were withdrawn from each jar using disposable syringes after 0, 15, 30, 60, 120, 180, and 210 or 240 min. Gas samples were stored until the end of each set of incubations by pushing the syringe needles into rubber bungs. Syringe volumes were carefully adjusted to 500 μl and the samples were injected onto a gas chromatograph (ATI-Unicam 610) that

Table 1. Rates of ethylene production in different experimental treatments at 22°C under 75 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ irradiance, standardized to volume of sediment (cm^{-3}) or fresh weight of *Caulerpa taxifolia* tissue ($\text{g}^{-1} \text{fwt}$), as appropriate.

	Ethylene production (nmol h^{-1})		Normalizing factor
	Mean	$\pm\text{SD}$	
Cap Martin			
Sediment	0.138	0.084	cm^{-3}
<i>Caulerpa taxifolia</i>	0.143	0.060	$\text{g}^{-1} \text{fwt}$
Sediment + <i>C. taxifolia</i>	3.960	1.985	cm^{-3}
Sediment + <i>C. taxifolia</i>	3.546	3.685	$\text{g}^{-1} \text{fwt}$
Monaco			
Sediment	0.106	0.045	cm^{-3}
<i>C. taxifolia</i>	0.097	0.071	$\text{g}^{-1} \text{fwt}$
Sediment + <i>C. taxifolia</i>	0.128	0.095	cm^{-3}
Sediment + <i>C. taxifolia</i>	0.004	0.002	$\text{g}^{-1} \text{fwt}$

had been precalibrated against a standard mixture of ethylene (0.5%) and acetylene. Samples were separated on a stainless steel column (1 m long; 2 mm diameter) filled with PORAPAK (80–100 mesh). Mean rates of acetylene reduction were calculated by fitting straight lines through the data after ethylene began accumulating in the headspace gas.

At the end of an assay, the weights of the sediments and algal tissues in each jar were determined. Sediment samples were then dried to constant weight at 120°C, and their volumes were determined by water displacement, hence enabling determination of the ratio of algal biomass to sediment dry weight or volume. Ethylene production rates were determined in triplicate for (1) control samples (seawater blanks), (2) dead sea grass sediments from Cap Martin, (3) free-living *C. taxifolia* samples from Cap Martin, (4) *C. taxifolia* samples rooted in dead sea grass sediments from Cap Martin, (5) sediments close to the Monaco sewer, (6) free-living *C. taxifolia* samples from close to the Monaco sewer, and (7) *C. taxifolia* rooted in sediments from close to the Monaco sewer.

Oven-dried (70°C) sediment samples from Cap Martin and *C. taxifolia* tissues from both Cap Martin and Monaco ($n = 5$ in each case) were additionally sent as part of a different study to the University of Colorado for C:N analysis. Algal samples were ground to a powder in a Wiley Mill; sediments were ground for 24 h using a roller-table grinder. The powdered sediment samples were divided into three parts. The first part was analyzed directly to obtain whole-sediment estimates of percent C and percent N. The second part was treated with 10% HCl to remove carbonates, the bulk inorganic constituent of sediments in the region, until there was no further evolution of CO_2 bubbles and then dried at 60°C. The remaining part was placed in a muffle furnace at 500°C for 3 h to determine the organic content by weight change after combustion. The percentages of C and N in algal tissues, untreated sediments, and the organic fractions of sediments were then determined by combusting samples at 1,000°C in a Carlo Erba NC 2100 elemental analyzer and then introducing the purified gases of N_2 and CO_2 via a

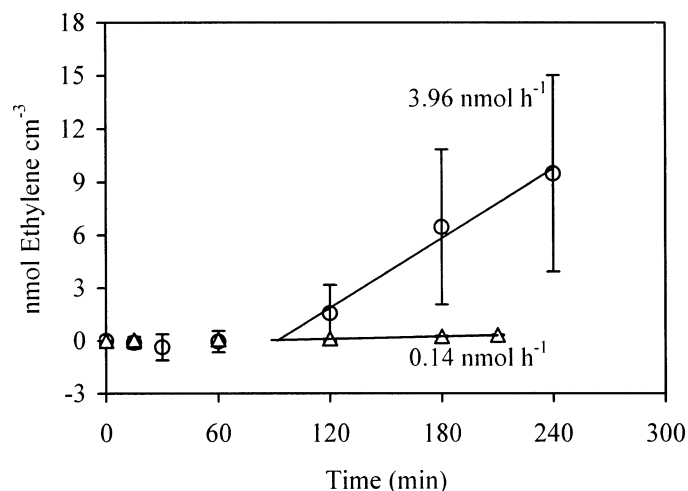


Fig. 3. Rates of acetylene reduction by bacteria in dead *Posidonia oceanica* sediments associated with *Caulerpa taxifolia* (circles) versus rates in uncolonized sediments (triangles) under 75 $\mu\text{mol m}^{-2} \text{s}^{-1}$ irradiance at a seawater temperature of 22°C. Open symbols indicate mean concentrations of ethylene; solid lines are straight line fits to the mean data after ethylene began appearing in the head gas. Their respective slopes are given adjacent; error bars are standard deviations.

stream of pure helium into a Thermo-Finnigan Delta Plus XL mass spectrometer.

Results

Whole-sediment samples from Cap Martin had an average C:N ratio of 40.67 (± 5.48 , SD) to 1 and a mean organic content of 29.7% ($\pm 6.86\%$). The organic component had a mean C:N ratio of 22.12 (± 6.26) to 1. Treatment with HCl has been shown to increase the apparent C and N content of leaves of the sea grass *Enhalus acoroides* by 7.6 and 14.2%, respectively, thus decreasing the C:N ratio by 5.7% (Bunn et al. 1995). If a similar error is present in our data, the mean organic C:N ratio of Cap Martin sediments would rise to 23.38:1, assuming a negligible contribution from any inorganic compounds not removed by acid treatment. The coefficients of variation ($\text{CV} = \text{SD}/\text{mean} \times 100$) for total organic matter content and its C:N ratio were 23.13 and 28.29%, respectively, indicating heterogeneity in both the quantity and quality of organic matter in sediments at Cap Martin.

Tissue samples of *C. taxifolia* from Cap Martin contained significantly less N than samples from Monaco: C:N = 14.14 \pm 1.08 versus 10.74 \pm 1.34 (t -test, $P = 0.0023$), indicating lower N or higher C supply rates of either inorganic or organic composition (see Chisholm et al. 1996).

Among the listed acetylene reduction treatments, the only samples that exhibited appreciable rates of ethylene production were dead sea grass sediments colonized by *C. taxifolia* from Cap Martin (Table 1). Acetylene reduction followed first-order rate kinetics following an initial lag of 60–90 min that presumably represented the time taken for the substrate gas to diffuse to the sites of reduction and for the product to evolve into the headspace of the jar (Fig. 3). There was

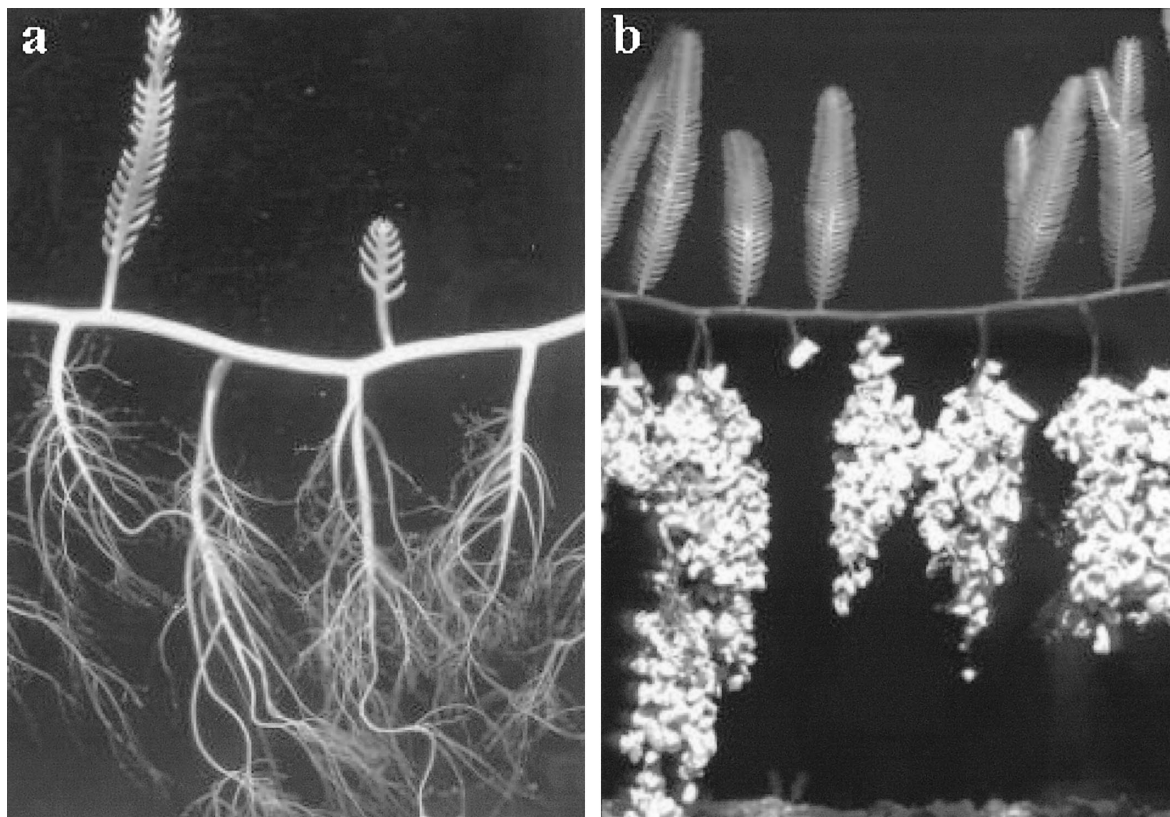


Fig. 4. Abundance of rhizoids relative to fronds and stolons in two cultured samples of *Caulerpa taxifolia*: (a) profuse growth of rhizoids in the absence of basal sediment and (b) variable growth of rhizoids in a bed of coarse carbonate sand (grains adhering to rhizoids) containing nonuniformly distributed organic matter.

no significant production of ethylene in the seawater control samples (linear regression analysis, slope not significantly different from zero, $P = 0.974$).

C. taxifolia increased acetylene reduction in Cap Martin sediments on average by a factor of 28, to yield a mean ethylene production rate of $3.96 \text{ nmol cm}^{-3} \text{ h}^{-1}$ ($=3.6 \text{ nmol g}^{-1}$ dry weight of sediment h^{-1} ; hereafter abbreviated to $\text{nmol g}^{-1} \text{ dws h}^{-1}$). The individual rates of acetylene reduction were highly variable: 2.5, 3.2, and $6.2 \text{ nmol ethylene cm}^{-3} \text{ h}^{-1}$ at algal fresh weight to sediment volume ratios of 1.9, 0.4, and 4.1 to 1, respectively.

We tested for significant differences in the acetylene reduction rates of the treatment groups using Welch's robust analysis of variance (Welch 1951), after decoupling the within-group variances from their means by natural logarithmic transformation. Welch's robust analysis of variance was used because there are no satisfactory tests for homogeneity of variance at very small sample sizes (Day and Quinn 1989). In the first instance, we compared the rates of acetylene reduction by the four sample groups that contained sediment, normalized to sediment volume; thus, Cap Martin and Monaco sediments, each with and without cover of *C. taxifolia*. In the second instance, we compared the rates of acetylene reduction by the four sample groups that contained *C. taxifolia*, normalized to algal fresh weight; thus, Cap Martin and Monaco *C. taxifolia* samples, each with and without sedi-

ment. Both tests revealed significant differences among the sample groups ($F_{3,4,3} = 29.6$, $P = 0.0025$ for the sediment comparisons; $F_{3,4,2} = 30.8$, $P = 0.0026$ for the algal comparisons).

Post hoc, pairwise comparisons of the log-transformed means indicated that sediments colonized by *C. taxifolia* at Cap Martin reduced acetylene at significantly faster rates than the other sample groups, whose rates did not differ significantly from one another (Student's t -tests, $P < 0.05$). Thus, there was no apparent stimulation of acetylene reduction in sediments close to the Monaco sewer outlets by *C. taxifolia*, either because water column N loads suppressed nitrogenase activity or because sediments lacked significant quantities of organic matter.

Discussion

Although the highest rate of acetylene reduction among Cap Martin sediments was measured on the sample with the largest algal biomass to sediment ratio, normalizing to total algal biomass did not substantially reduce the variability of the data. Total algal biomass might not be a good proxy for rhizoid surface area or activity within the sediment, as the overwhelming bulk is composed of aerial fronds and stolons. Indeed, rhizoid abundance might be negatively correlated to aerial biomass when nutrients are in short supply (Fig. 4).

We also suggest that heterogeneity in the sediment distribution of *P. oceanica* tissues of differing C:N ratio likely influences the distribution of microbial populations (see Results, CV for content and C:N ratio of organic matter). Thus, as *C. taxifolia* sends down rhizoids at regular intervals along its stolon, chance largely determines which of those rhizoids tap "favorable" pockets of sediment.

Unfortunately, we were unable to quantify rhizoid abundance within the sediment, and we did not investigate the composition or activity of microbial populations surrounding individual rhizoid clusters. Thus, although our data suggest that *C. taxifolia* substantially enhances N_2 fixation in high C:N organic sediments, we recognize that many more measurements are needed to reliably estimate the overall degree of enhancement.

More than 90% of the total cover of *C. taxifolia* in the Mediterranean is found between Toulon in France and Genoa in Italy (Meinesz et al. 2001). The alga is not distributed uniformly within this zone. Most of the heavy development has occurred in the neighborhood of poorly treated wastewater discharges, often upon decaying beds of *P. oceanica* (Chisholm et al. 1997). *P. oceanica* has regressed in the northern Mediterranean for the last several decades, ostensibly as a result of pollution and coastal development (e.g., Pérès and Picard 1975; Meinesz and Laurent 1978; Falconetti and Meinesz 1989).

P. oceanica produces a complex network of rhizomes, which supports growth of foliage and development of the root system. Under favorable conditions, *Posidonia* beds can grow for tens to hundreds of years and form massive banks of accumulated organic material. Much of this material is structural and, thus, principally composed of carbon, hydrogen, and oxygen. The N:C ratios of whole sea grass tissues are typically very low, 1:17 or less (e.g., Paerl 1990; Kristensen and Hansen 1995; data herein), whereas phytoplankton have a mean N:C content of about 1:6.6 (Redfield et al. 1963). Because bacteria typically require larger amounts of N than eukaryotic cells, their ability to decompose dead sea grass tissues is compromised by nitrogen deficiency (Goldman et al. 1987). Data provided here indicate that *C. taxifolia* exploits this situation by stimulating a series of microbial reactions that culminate in the fixation of N_2 . N_2 fixation can alleviate limitations on bacterial growth, thereby accelerating the transformation of organic waste (e.g., Paerl 1990; Tibbles et al. 1994). Because bacterial turnover usually results in the production of low-molecular-weight organic compounds, CO_2 , inorganic P, and trace elements, in addition to inorganic N, the primary benefit to *C. taxifolia* need not in fact be nitrogen.

Although excretion of photosynthetic products into the substratum formerly has been described in saltwater vascular plants (e.g., Pollard and Moriarty 1991; Welsh et al. 1997; Blaabjerg et al. 1998), to the best of our knowledge, this is the first time that the process has been described in a macroalga. This may well be a consequence of the fact that most algae require consolidated substrata for attachment and thus do not excrete photosynthetic products into soft bottom sediments. It would be interesting to examine whether other rhizophytic algae, such as *Halimeda*, which forms extensive meadows in oligotrophic tropical waters, also stimulate nu-

trient delivery by releasing photosynthetic product into the substratum.

Although we have not identified the specific products that *C. taxifolia* releases into its rhizosphere, they are likely simple sugars (see Tibbles et al. 1994; Welsh et al. 1997). Releasing carbon compounds that contain significant amounts of limiting nutrients would be counterproductive. Moreover, the black, sulfide-rich zone that forms around the rhizoids of *Caulerpa* can be reproduced by injecting solutions of glucose or sucrose into unvegetated sediments (unpubl. data).

Several physicochemical parameters alter concomitantly with formation of the black sulfide zone. Redox potential oscillates diurnally as the rhizoids grow into the substratum (Fig. 5a), likely accelerating organic matter decomposition (e.g., Aller 1994). In the light, temperature rises in the rhizosphere relative to unvegetated sediments, consistent with an increase in microbial respiration (Fig. 5a). The pH falls progressively toward 6, probably mostly because of hydration of respired CO_2 , whereas oxygen concentration rises slightly during algal photosynthesis, possibly promoting microaerophilic N_2 fixation close to the alga's rhizoids (Fig. 5b; see Tibbles et al. 1994 for discussion and references).

The biochemical reactions driving these changes are of variable duration, lasting from a few to many days (see, e.g., Fig. 5a, redox oscillation). They are also of varying magnitude, likely reflecting changes in the photosynthetic yield of the alga, age and activity of the rhizoids, nature and abundance of substrates present in the sediment, and heterogeneity in the resident microbial communities. These factors likely contribute to the measured variability in rate of acetylene reduction, which appears neither strongly correlated with frond and stolon biomass nor total sediment volume. Moreover, although *Caulerpa* exhibits a highly regular pattern of growth, the capacity of different rhizoids to stimulate sulfate reduction is variable. The stolons of *Caulerpa* exhibit a fortuitous tendency to grow along the sidewalls of glass aquaria, providing an excellent opportunity to observe the build-up of sulfide in successive rhizoid clusters (e.g., Fig. 1). Although the youngest rhizoid cluster, the one closest to the stolon apex, is usually more active, older rhizoid clusters further from the apex show varying degrees of sulfide build-up that are not always directly related to the age of the rhizoids. One might speculate that positive feedback exists, whereby *Caulerpa* invests more of its photosynthetic yield in sediments that provide high rates of nutrient delivery.

It is generally believed that anaerobic decomposition of organic waste in marine sediments commences with hydrolytic fermentation, which yields secondary substrates to the sulfate-reducing bacterial community (Westrich and Berner 1984; Kristensen and Hansen 1995). Sulfate reducers control phosphorus release within sediments creating reducing conditions that favor nitrogen fixation (see Pollard and Moriarty 1991 and references therein). Because many sulfate reducers also fix N_2 , we assume that these bacteria were responsible for the bulk of the measured ethylene production (see Welsh et al. 1996a,b).

Our data permit only a very approximate estimation of the rates of N_2 fixation that might occur in the field as a consequence of *C. taxifolia* development. First, the stoichiometry of acetylene reduction to N_2 fixation can depart sub-

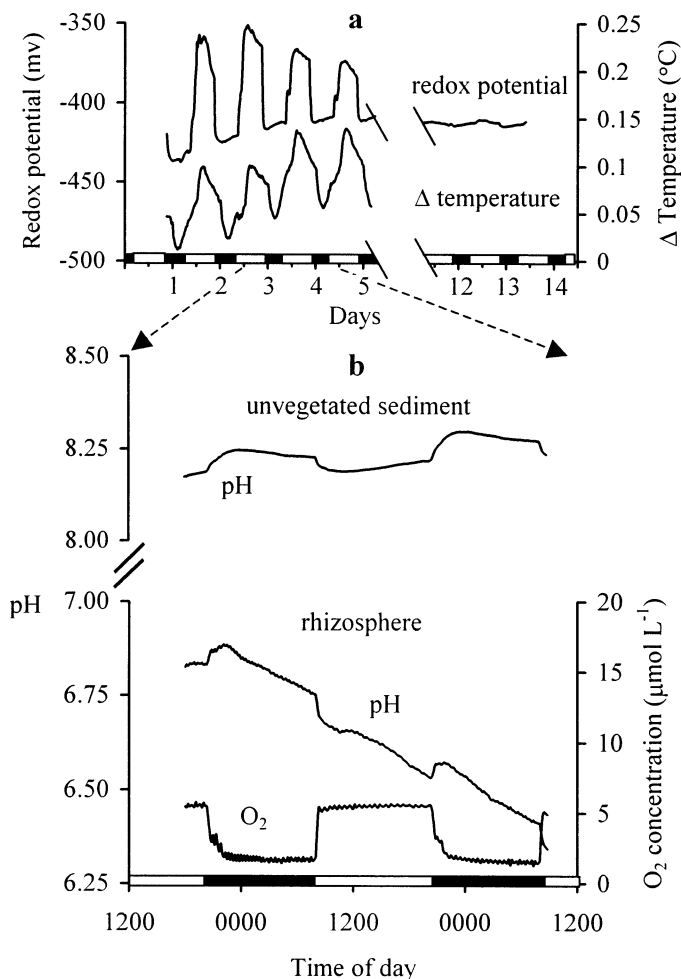


Fig. 5. Example profiles of changes in physicochemical parameters in the rhizosphere of *Caulerpa taxifolia* shortly after penetration of high C:N organic sediments by its rhizoids: (a) redox potential within a rhizosphere and difference in temperature between a rhizosphere and adjacent unvegetated sediment and (b) pH change in unvegetated sediment in the upper part of the figure compared with pH change in an adjacent rhizosphere in the lower part of the figure, concomitant with rhizosphere changes in oxygen concentration. All measurements were made at a depth of 15 mm below the sediment horizon in aquaria containing seawater at $22 \pm 0.5^{\circ}$ C; white and black bars on abscissas indicate light (100μ mol photons $m^{-2} s^{-1}$) and dark periods, respectively. Redox measurements were made with a Lazar ORP-146C Micro Combination Electrode, calibrated using Schott standards (+190 mV, +430 mV), connected to a Radiometer PHM85 meter. Differential temperature was measured with a pair of Teflon-insulated Physitemp micro thermocouples, inserted to the extremities of glass pasteur pipettes for protection, connected to a Physitemp BAT-10 meter. The pH measurements in unvegetated sediment were made with a Ross-Orion 8103 Semi-Micro Combination Electrode connected to a Radiometer PHM85 meter. Rhizosphere pH and oxygen measurements were made with a Diamond General 757 Needle Combination Oxygen/pH Electrode connected via an isolation amplifier to a Diamond General Chemical Microsensor. Both pH electrodes were calibrated against Radiometer buffer solutions of 4.01, 7, and 9.18 nominal pH units at 25° C; the oxygen sensor was calibrated against air-saturated seawater and a saturated solution of sodium sulfite. All data were recorded at 10-min intervals using Li-Cor 1000 data loggers.

stantially from the theoretical ratio of 3:1 (see e.g., Howarth et al. 1988). Second, although we endeavored to minimize disturbance of the sediment samples and the sediment–algal associations, to a certain extent this was unavoidable during transfer of the samples into the incubation vessels. Third, because photosynthesis in *C. taxifolia* at a depth of 10 m is light saturated at around $200\text{--}300 \mu$ mol photons $m^{-2} s^{-1}$ (Chisholm and Jaubert 1997) and we provided only 75μ mol photons $m^{-2} s^{-1}$ during assays, the flow of labile photosynthetic product into the sediment was likely subordinate to that which occurs at an equivalent depth in situ.

Nevertheless, the mean rate of ethylene production by dead *P. oceanica* sediments associated with *C. taxifolia* at Cap Martin of $3.6 \text{ nmol } g^{-1} \text{ dws } h^{-1}$ is nearly five times the maximum rate reported for sediments associated with the sea grass *Zostera capensis* (Tibbles et al. 1994). Tibbles et al. (1994) found that acetylene reduction rates were strongly influenced by the quantity of organic matter in the sediment and, to varying degrees, by bioturbation, oxygen tension, and the types of organic substrates available to the microbial community. We do not know why sediments associated with *C. taxifolia* should exhibit such high rates of acetylene reduction relative to *Z. capensis* sediments, but we presume that a much smaller amount of organic material is available to heterotrophic bacteria in sediments supporting living populations of *Zostera* than in decaying beds of *P. oceanica*.

Notwithstanding the many limitations of our data, we offer the following conjecture. Standardizing our acetylene reduction rates to fresh weight of *Caulerpa* tissue (fwt) yielded a mean ethylene production rate of $3.5 \text{ nmol } g^{-1} h^{-1}$. The year-round biomass of *C. taxifolia* at Cap Martin has been estimated at $5.5 \text{ kg fwt } m^{-2}$ (Meinesz et al. 1995). By crudely assuming that our measured rate is constant over time and that 3 moles of acetylene are reduced for every mole of N_2 fixed, which appears reasonable for marine sediments colonized by macrophytes (Howarth et al. 1988), we estimate an annual contribution to N production of $1.6 \text{ g } m^{-2}$ for heavily colonized sediments. According to data reviewed by Howarth et al. (1988), this rate significantly exceeds most other short-term estimates of N_2 fixation by heterotrophic bacteria in sediments collected from similar environments.

Chisholm and Jaubert (1997) estimate that *C. taxifolia* fixes close to $4 \text{ g } C \text{ m}^{-2} \text{ d}^{-1}$ (net photosynthesis) at 10 m depth and similar seawater temperatures ($23\text{--}24^{\circ}$ C) to that used in this study (22° C). Such production translates to a dry weight (dwt) increase of $7.03 \text{ g } m^{-2} \text{ d}^{-1}$ using a mean organic C to total dry weight ratio of 1.757:1 (Gacia et al. 1996). By crude calculation, production of an additional $7.03 \text{ g fwt } m^{-2} \text{ d}^{-1}$ of algal tissue could stimulate $55 \text{ mg } m^{-2} \text{ d}^{-1}$ of new N production in the underlying sediment (see Fig. 6). *C. taxifolia* requires 285.7 mg of N to produce $4 \text{ g } C \text{ m}^{-2} \text{ d}^{-1}$ at a C:N ratio of 14:1 (see data for Cap Martin samples). Thus, at the rates measured here, N_2 fixation could satisfy approximately 19% of the alga's N requirement in the unlikely event that 100% of the fixed N was transferred to alga. Although 19% of the total N requirement is far from insignificant and might be substantially higher in situ, a substantially greater amount of N must nonetheless come from existing inorganic reservoirs in the sediment or water column, or from turnover of organic matter.

Production of $3.5 \text{ nmol C}_2\text{H}_4 \text{ g}^{-1} \text{ fwt h}^{-1} \approx 1.17 \text{ nmol N}_2 \text{ g}^{-1} \text{ fwt h}^{-1}$ (i.e., 3:1) = $2.33 \text{ nmol N g}^{-1} \text{ fwt h}^{-1}$
 $\Rightarrow 2.33 \text{ nmol N g}^{-1} \text{ fwt h}^{-1} \times 24 \text{ h} = 56 \text{ nmol N g}^{-1} \text{ fwt d}^{-1}$
 $\Rightarrow 56 \text{ nmol N g}^{-1} \text{ fwt d}^{-1} \times 14 \text{ g (atomic weight of N)} = 784 \text{ ng N g}^{-1} \text{ fwt d}^{-1}$
 $\Rightarrow 784 \text{ ng N g}^{-1} \text{ fwt d}^{-1} \times 10 \text{ (wet to dry weight ratio of alga)} = 7.84 \text{ mg N g}^{-1} \text{ dwt d}^{-1}$
 $\Rightarrow 7.84 \text{ mg N g}^{-1} \text{ dwt d}^{-1} \times 7.03 \text{ g dwt m}^{-2} \text{ d}^{-1}$ (i.e., algal dry weight production) = $55 \text{ mg N m}^{-2} \text{ d}^{-1}$

Fig. 6. Coarse estimation of new N production in high C:N organic sediments associated with net production of $4 \text{ g C m}^{-2} \text{ d}^{-1}$ by *Caulerpa taxifolia*.

It is likely that heterotrophic bacteria are the primary beneficiaries of N_2 fixation, as new N production would enable them to degrade otherwise refractory organic compounds, thereby remineralizing C, N, and P. By corollary, as the transformation of sediment organic matter approaches completion, the abundance of *Caulerpa* should decrease dramatically in the absence of an exogenous N supply of similar magnitude. Might this not explain some of the many boom-bust cycles of *Caulerpa* growth that have occurred in different environments (Ollivier 1929; Rayss 1941; May 1976; LaPointe et al. 1994; Panayotidis and Montesanto 1994; Doumenge 1995; Davis et al. 1997)?

Our data indicate that growth of *C. taxifolia* substantially enhances nitrogen production in high C:N sediments, thus facilitating organic matter turnover. Accelerated turnover would help to explain how *C. taxifolia* can develop a biomass of $\sim 4 \text{ kg fwt m}^{-2}$ or more (Verlaque and Fritayre 1994; Meinesz et al. 1995) on dead *Posidonia* sediments in relatively low-nutrient seawater. *C. taxifolia*'s ability to stimulate nutrient turnover in substrata and then take up a proportion of the resulting nutrients via its subterranean rhizoids likely assists the remediation of sediments that are burdened by large quantities of refractory organic waste.

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